Developmental Biology **232,** 362–371 (2001)
doi:10.1006/dbio.2001.0175, available online at http://www.idealibrary.com on IDEAL®

Development and Evolution of a Variable Left–Right Asymmetry in Nematodes: The Handedness of P11/P12 Migration

Marie Delattre and Marie-Anne Félix

Institut Jacques Monod, CNRS, Universités Paris 6 and 7, Tour 43, 2 Place Jussieu, 75251 Paris Cedex 05, France

In *Caenorhabditis elegans*, two lateral blast cells called P(11/12)L and P(11/12)R are symmetric left–right homologs at hatching, migrate subsequently in opposite anteroposterior directions during the first larval stage, and adopt two different fates, thus breaking the symmetry between them. Our results show that, unlike most other cell fate decisions in *C. elegans*, the orientation of P(11/12)L/R migration is highly biased, but not fixed. The handedness of their migration is linked to whole body handedness and is randomized in *lin-12*/Notch mutants and by ablation of the Y cell. Migration handedness is independent of P11 and P12 fate determination, previously shown to require the LIN-44/Wnt and the LIN-3/EGF pathways (L. I. Jiang and P. W. Sternberg, 1998, *Development* 125, 2337–2347). We further show that several changes in P(11/12)L/R asymmetry have occurred during nematode evolution: loss of asymmetry or reversals in orientation of migration. Strikingly, for most species studied, handedness of migration is highly biased but not fixed. Thus, whereas the final cell fate pattern of P11/12 is invariant, the developmental route leading to it is subject both to developmental indeterminacy and to evolutionary variations.

Key Words: **left–right asymmetry; nematodes; evolution; LIN-12/Notch; handedness; cell lineage; P11/P12.**

INTRODUCTION

Like other Bilateria, nematodes are mostly symmetric between the left and the right sides of the body, but exhibit some residual asymmetries. Here we report on the study of a left–right asymmetry between two lateral blast cells called P(11/12)L and P(11/12)R.

At hatching in *Caenorhabditis elegans*, 12 ventral epidermal blast cells, the Pn cells, are positioned along the anteroposterior axis in six left–right pairs (Fig. 1A). During the first larval (L1) stage, they rotate and become aligned along the anteroposterior axis in the ventral cord (Sulston and Horvitz, 1977). Under Nomarski optics, this rotation is seen as a ventral migration of Pn cell nuclei, coming from the left and right sides of the body. The left and right cells of a Pn pair contact each other on their ventral side and remain in contact during rotation (Sharma-Kishore *et al.*, 1999) (Fig. 1).

For the most posterior pair, P(11/12)L designates the left cell before migration, P(11/12)R the right cell. After migration, the anterior cell is called P11, the posterior cell P12 (Sulston and Horvitz, 1977). The two cells are symmetric left–right homologs in the embryonic lineage (Sulston *et al.*, 1983). For most pairs, the rotation occurs in either direction with the same probability. However in *C. elegans*, rotation is biased for the P1/2 and P11/12 pairs. In the latter case, Sulston and Horvitz (1977) reported that in 7/7 animals, P11 came from the left and P12 from the right. P11 and P12 then adopt two different fates (Fig. 1B). Like the other Pn cells, they divide and give rise to a Pn.a cell (neuroblast) and a Pn.p cell. P11.a and P12.a give rise to different types of neurons; P11.p fuses with the epidermal syncytium hyp7, like more anterior Pn.p cells, whereas P12.p divides once more and one daughter contributes to the rectum (Fig. 2C) (Sulston and Horvitz, 1977).

The P(11/12)L/R blast cells can be found in the L1 larva of other nematode species and they adopt two different fates as in *C. elegans*. Sternberg and Horvitz (1982) have shown that in *Panagrellus redivivus* as in *C. elegans*, P cell rotation is not random for the P1/2 and P11/12 pairs. However, in *P. redivivus*, the anterior cell P11 comes from the right side of the body, in contrast to *C. elegans*. This homology of the cells between species and the variation described in *Panagrellus* prompted us to undertake an

0012-1606/01 $35.00

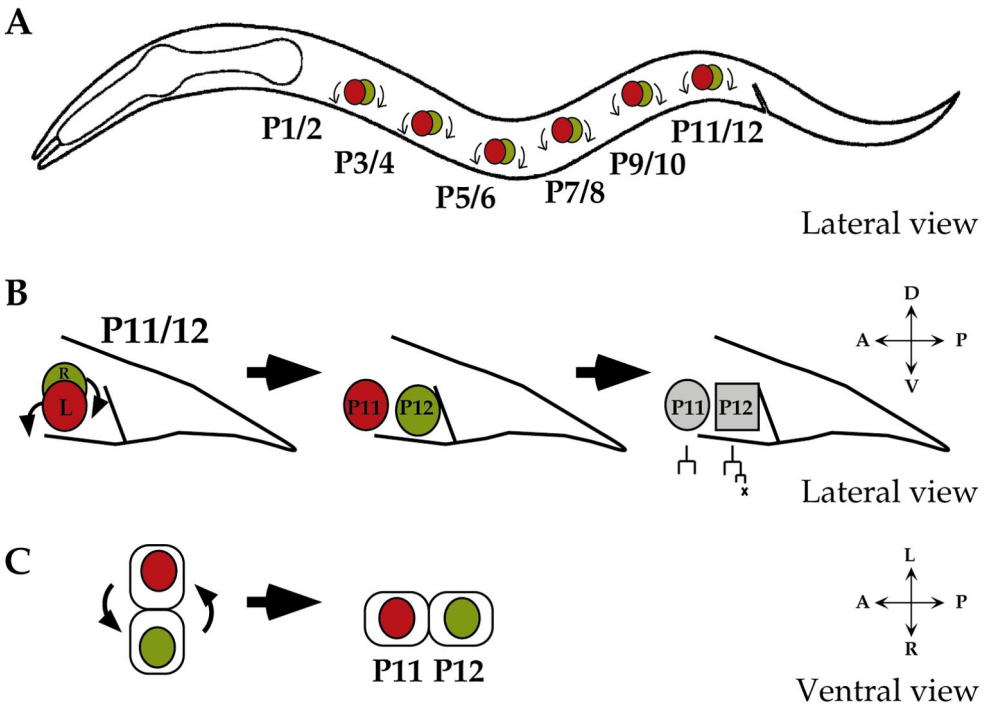

FIG. 1. Migration of the two left–right asymmetric cells P(11/12)L and P(11/12)R. (A) At hatching, six pairs of Pn cells are located on either side of the body. During the first larval stage, the nuclei of these cells (circles) enter the ventral cord and align along the anteroposterior axis. The anterior cell is then called P1 and the posterior cell P12 (Sulston and Horvitz, 1977). (B) Lateral views of P(11/12)L/R nuclei (circles). The P11/12 pair is located immediately anterior to the rectum. After their migration, the two cells divide and their posterior daughters (P11.p and P12.p) adopt two different fates, characterized by their lineage (the lineage of the anterior daughters is not shown). Left side is in red, right side is in green. P11 and P12 fates are represented by a circle and a square, respectively. (C) Ventral views of the cells contacting each other during rotation. Cell shape is highly schematic.

evolutionary comparison of the behavior of this cell pair. We then wondered how the asymmetry in migration between these two cells is established during development and whether the handedness of their migration is linked with their distinct fate determination.

We show that the chirality of migration and the final cell fate determination of P11/12 are controlled by distinct mechanisms in *C. elegans* and that the former requires the presence of the Y cell and is linked to whole body handedness. We also show that the orientation of migration is highly biased but not fixed in *C. elegans*, nor in any of the species which we studied, and that it has switched polarity several times during evolution. In addition, it appears fully random in some species. Thus, although all species have the same final pattern of P11 and P12 fates, the handedness of migration of P(11/12)L/R is not fixed in the development of a given species and is subject to evolutionary changes.

MATERIALS AND METHODS

Strains and Culture Conditions

The nematode species listed in Fig. 3 have been kindly provided by L. Carta, D. Fitch, P. De Ley, J. Baldwin, W. Sudhaus, and the *C. elegans* Genetics Center. We did not study species of the Diplogastridae, another family of free-living soil nematodes (such as *Pristionchus pacificus*), because migration of the Pn cells occurs in the late embryo before hatching and is therefore difficult to follow in these species.

N2 (Bristol) strain was used as wild-type *C. elegans*. The following mutant alleles were used.

- LG I: *lin-17(n671)* (Ferguson and Horvitz, 1985), *lin-44(n1792)* (Herman and Horvitz, 1994).
- LG III: *ncl-1(e1865)* (Hedgecock and Herman, 1995), *egl-5(n486)* (Trent *et al.*, 1983), *unc-36(e251)*, *dpy-19(e1259)*, *unc-32(e189)* (Brenner, 1974), *lin-12(n137)*, *lin-12(n941)*, *lin-12(n137n720)*, *lin-12(n676n930)*, *lin-12(n676n909)*, *qDp3* (Austin and Kimble, 1987). *lin-12(n137)* is a semidominant gain-of-function allele (Greenwald *et al.*, 1983). *n941* is a putative null allele (Wen and Greenwald, 1999). *n137n720* and *n676n909* are reduction-of-function alleles (Seydoux *et al.*, 1990). *n676n930* is a temperature-sensitive reduction-of-function allele (Sundaram and Greenwald, 1993).
- LG IV: *unc-24(e138)* (Brenner, 1974), *lin-3(n378)*, *lin-3(n1059)* (Ferguson and Horvitz, 1985), *let-59(s49)* (Rogalski and Baillie, 1985), *dpy-20(e1282)* (Brenner, 1974), *unc-22(s7)* (Moerman and Baillie, 1979). *lin-3(n378)* is a reduction-of-function allele. *lin-3(n1059)* is a lethal loss-of-function allele (Ferguson and Horvitz, 1985; Liu *et al.*, 1999).

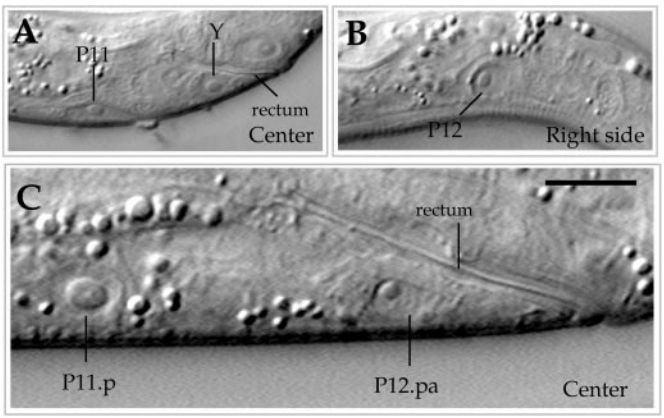

FIG. 2. Nomarski photomicrographs of P11/12 development. (A) *C. elegans* L1 larva, sagittal view. The left cell nucleus has migrated into the ventral cord. The Y cell nucleus is on the midline. (B) Right lateral view of the same animal. The right cell nucleus is still on the right side, posterior to its left counterpart. This configuration corresponds to the orientation of P11/12 migration in most wild-type *C. elegans* larvae: P11 comes from the left and P12 from the right. (C) P11.p and P12.pa have two different morphologies in a L4 larva, which serve as markers of P11/12 fates. Scale bar, 10 μm.

• LG V: *lag-2(q420)* (Lambie and Kimble, 1991), *him-5(e1490)* (Hodgkin *et al.*, 1979). *lag-2(q420)* is a temperature-sensitive reduction-of-function allele.

• LG X: *bar-1(sy324)* (A. Golden, L. Jiang, and P. Sternberg, pers. comm.).

The following strains were used.

• MT1306 *lin-17(n671).* PS2308 *lin-44(n1792); lin-3(n378) let-59(s49) unc-22(s7)/unc-24(e138) lin-3(n1059) dpy-20(e1282).* MT1081 *egl-5(n486).* CB189 *unc-32(e189).* MT688 *lin-12(n137)/unc-32(e189); him-5(e1467).* MT2375 *dpy-19(e1259) lin-12(n137)/unc-32(e189) lin-12(n676n909); him-5(e1490).* GS67 *ncl-1(e1865) unc-36(e251) lin-12(n941); qDp3.* MT2343 *lin-12(n137) dpy-19(e1259)/lin-12(n137n720) unc-32(e189).* GS60 *unc-32(e189) lin-12(n676n930).* JK1277 *lag-2(q420).* PS1489 *bar-1(sy324).*

• JU105: The *sy466* mutant is a partially sinistral mutant of the *Oscheius/Dolichorhabditis* sp. CEW1 (the genus name of this species is currently under revision; L. Carta, pers. comm.; see Félix *et al.*, 2000a). This mutant was isolated in a random F2 screen after EMS mutagenesis of the CEW1 strain on the basis of its partially penetrant egg-laying defective phenotype. It was outcrossed five times to wild type and the temperature-sensitive sinistral phenotype cosegregated with the following phenotypes: enlargement of the vulval competence group to P3.p, egg-laying defective (partial penetrance), and gonadal defects (temperature-sensitive). The proportions of sinistral animals in wild-type CEW1 and *sy466* animals are 0/200 (0%) and 4/96 (4%) at 20°C, 0/100 (0%) and 28/230 (12%) at 25°C, and 0/39 (0%) and 67/348 (19%) at 28°C, respectively. The animals were raised at 25°C for observations of P11/12 migration, because sterility was high at 28°C. Among the left–right asymmetries defined in Sulston and Horvitz (1977), we checked that the positions of the gonad, ventral nerve cord, coelomocytes, M cell, and sphincter muscle were inverted in sinistral compared to dextral *sy466* animals.

• Standard media and methods for culturing and breeding were as described in Sulston and Hodgkin (1988). Most strains were grown at 20°C. GS60 and JU105 were grown at 25°C (unless otherwise indicated). JK1277 was grown at 18°C; gravid hermaphrodites were shifted at 25°C and P11/12 migration was followed in their progeny. All microscopic observations were performed at room temperature.

Temperature-Shift Experiment of the lin-12(ts) Allele

The *lin-12(n676n930)* allele is a temperature-sensitive allele (Sundaram and Greenwald, 1993). At 15°C, 70% of L1 larvae have a Y cell (43/60) and 30% have no Y cell (17/60), whereas animals grown at 25°C (restrictive temperature) have no Y cells (42/42). In comparison, wild-type animals grown at 25°C always have a Y cell (61/61). For the shift experiment, the GS60 strain was cultured at 15°C. Embryos that were born at 15°C were placed at 25°C. Two hours later, larvae that had already hatched were killed so that the animals which were later observed for P11/12 migration had been shifted at 25°C at least 2 h before hatching. Thus, 70% of these animals have a Y cell at the time of P11/12 migration but the migration occurs in the absence of a functional LIN-12.

Cell Nomenclature

Once the 12 Pn cells are aligned along the anteroposterior axis of the mid-L1 larva, they are named P1 to P12 from anterior to posterior. Earlier in the L1 stage, they are positioned in six left–right pairs. For the most posterior pair, P(11/12)L designates the left cell of the pair, P(11/12)R the right cell, whereas P(11/12)L/R designates the pair before migration. P11 and P12 designate these cells after migration, as well as their distinct fates. P11/12 is used for the pair in general, either before (if not ambiguous) or after migration.

Microscopy

Mid-L1 stage larvae were mounted on agar pads as described in Sulston and Hodgkin (1988), and P11/12 migration was observed using a Zeiss Axioskop with 100× Nomarski optics. Migration of the Pn cells usually follows an anteroposterior chronology, with P1/P2 migrating first and P11/P12 last. Within the P11/12 pair, the order of P11/12 migration is not fixed: P11 migrates first in 19/31 wild-type *C. elegans*. In *C. elegans*, the entire migration of all Pn cells from P1 to P12 takes half an hour to 1 hour. For some species, especially in the Cephalobidae family, it lasts only 5 min.

For balanced mutant strains, the genotype of the animal was later checked in the adult stage. In this case, the L1 larva was transferred onto a fresh agar plate shortly after P11/12 cell migration and allowed to grow until adulthood.

The final P11/P12 cell fate pattern was scored in the L4 stage by observing nuclear morphology of P11.p and P12.pa (see Fig. 2C and Jiang and Sternberg, 1998).

Cell ablation experiments were carried out on larvae picked 0–1 h after hatching, as described in Bargmann and Avery (1995).

TABLE 1
Orientation of P(11/12)L/R Migration and P11/P12 Determination Are Two Independent Processes

Genotype	Homolog	Migration P12 from the right	Determination P12 to P11 transformation
Wild type *C. elegans* N2		29/34 (85%)	0/many (0%)
	(A) *C. elegans* mutants affecting P11/P12 determination		
lin-17(*n671*)	Frizzled (lf)	11/12	11/41 (27%)[a]
lin-44(*n1792*); *lin-3*(*n378*)	Wnt; EGF (lf)	10/12	124/159 (78%)[a]
egl-5(*n486*)	AbdB (lf)	12/12	41/47 (87%)
bar-1(*sy324*)	Armadillo (lf)	20/24 (83%)	43/43 (100%)
	(B) Sinistral mutant in *Oscheius/Dolichorhabditis* sp. CEW1		
Wild type CEW1		24/28	
sy466, dextral, 25°C		30/40	
sy466, sinistral, 25°C		4/15	
	(C) *C. elegans* mutants affecting P11/12 migration		
lin-12(*n676n909*)	Notch (lf)	13/30 (44%)[b]	0/20 (0%)
lin-12(*n137n720*)	Notch (lf)	6/13	0/38 (0%)
lin-12(*n676n930ts*), 25°C	Notch (lf)	24/40 (60%)[b]	4/57 (7%)
lin-12(*n941*)	Notch (lf)	25/40 (62%)[b,c]	0/23 (0%)
lag-2(*q420ts*), 25°C	Delta (lf)	34/48 (71%)	

Note. The number of animals in which P(11/12)R migrated posteriorly and the number with a P12 to P11 fate transformation were determined for different genotypes. lf, loss-of-function allele. Most of the *lin-12* alleles were balanced because of sterility or lethality. As several strains were constructed using *unc-32*(*e189*) as a marker for the *lin-12* allele, we checked that this mutation did not affect P11/12 migration (12/15 animals with the right cell migrating posteriorly).

[a] Data from Jiang and Sternberg (1998).

[b] Significantly different from wild-type N2 (χ^2 test, $P < 0.05$).

[c] The loss of the free duplication *qDp3* that balances *lin-12*(*n941*) was scored as animals that were PVul and sterile; since these phenotypes have a cellular focus in the MS-derived gonad (Seydoux and Greenwald, 1989; Seydoux *et al.*, 1990), this set is likely to contain partial mosaics in which *lin-12*(+) is still present for the decision in P11/12 migration (Y/DA9 are descendants of ABp) and may therefore underestimate the effect of *lin-12*(*n941*).

RESULTS

The Orientation of P(11/12)L/R Migration Is Not Fixed

Sulston and Horvitz (1977) reported that in 7/7 *C. elegans* larvae, the left cell of the P(11/12)L/R pair migrated anteriorly and became P11, whereas the right cell migrated posteriorly and became P12. We observed the migration of P(11/12)L/R in additional *C. elegans* wild-type hermaphrodites. Surprisingly, the orientation of migration is not fixed. For 29/34 animals observed, P12 came from the right, but in the remaining 5 animals, it came from the left (Table 1). Thus, the lineage relationship between the left and the right cells of the pair and P11/P12 is not fixed, but highly biased (around 85%), whereas the final P11/P12 cell fate pattern is invariant.

Mutations Affecting P11/P12 Fate Determination Do Not Affect the Orientation of P(11/12)L/R Migration

In *C. elegans*, if P(11/12)R is ablated, the left cell adopts the P12 fate (Sulston and Horvitz, 1977). Thus, P(11/12)L and P(11/12)R are both competent to adopt the P12 fate. During embryogenesis, a Wnt signal LIN-44 renders both cells competent to receive a LIN-3/EGF determination signal, which in turn activates the Ras–MAP kinase pathway in the posterior cell, leading to expression of the Hox gene *egl-5*/Abd-B (Jiang and Sternberg, 1998). The cell expressing EGL-5 adopts a P12 fate. LIN-3 is expressed in some cells in the tail that lie posterior to the P11/12 pair (P. Sternberg, pers. comm.) and might act as an asymmetric signal activating the P12 fate in one of the cells only. We wondered whether the right cell usually moves posteriorly as a consequence of receiving the LIN-3 signal and adopting

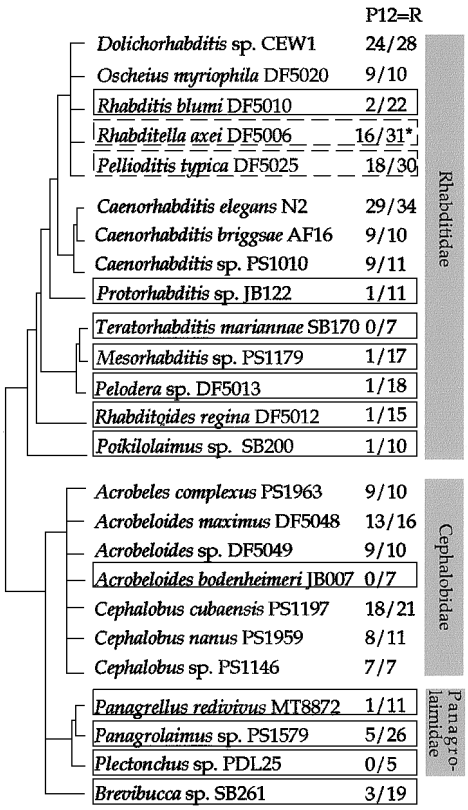

FIG. 3. Evolution of the handedness of P11/12 migration in the nematode order Rhabditida. The number of animals with the right cell migrating posteriorly is indicated for each species. Species for which P12 usually comes from the left are boxed with a full line. Those for which the migration appears to be unbiased are boxed with a dashed line. All individuals observed were dextral, except for *A. bodenheimeri.* The data for *P. redivivus* are from Sternberg and Horvitz (1982) (the animal with reversed P11/12 migration may have been sinistral). The phylogeny indicated on the left is based on Blaxter *et al.* (1998), Félix *et al.* (2000b), Sudhaus and Fitch (2001), and D. Fitch, personal communication. The relative positions of the three families and that of *Brevibucca* are uncertain (discussed in Félix *et al.*, 2000b). The favored topology (with the Cephalobidae and Panagrolaimidae in a common clade not comprising the Rhabditidae) is drawn here. *Brevibucca* (family Brevibuccidae) is classically classified close to the Panagrolaimidae (Andrassy, 1984). *In *Rhabditella axei,* the relative anteroposterior nuclear positions of P(11/12)L/R before migration (a good indicator of their subsequent pattern of migration) was scored in additional animals; this resulted in a total of 54/117 animals (46%) with the right cell moving posteriorly.

a P12 fate or if it adopts a P12 fate as a consequence of its posterior migration. We therefore determined the orientation of P(11/12)L/R migration in mutants affecting P12 determination: a *lin-44; lin-3* double mutant, a *lin-17* mutant (LIN-17 is the putative receptor of Wnt/LIN-44; Herman *et al.*, 1995; Sawa *et al.*, 1996), a *bar-1* mutant (BAR-1/Armadillo is an effector of the Wnt pathway; Eisenmann *et al.*, 1998), and an *egl-5* mutant. In all mutants observed, both cells of the P11/12 pair generally adopt the P11 fate (Table 1A). If the migration handedness was a consequence of fate determination, it should be unbiased in these mutants. However, we see a biased migration pattern of P(11/12)L/R similar to that of wild type (Table 1A). Thus, a left–right asymmetry between the two cells of the P11/12 pair still exists in mutants of their final fate determination.

Left–Right Asymmetry in the P(11/12)L/R Pair Is Linked to Whole Body Handedness

In *C. elegans,* the left–right asymmetry of the body plan is established at the six-cell stage (Wood, 1991). All individuals display the same body handedness defined as dextral (zur Strassen, 1951). The most striking asymmetry of the adult hermaphrodite is that of the gonad: the anterior gonadal arm lies on the right of the intestine, whereas the posterior arm is on the left. By micromanipulation of blastomeres at the six-cell stage, the orientation of left–right asymmetric cells can be inverted, giving rise to a sinistral animal (Wood, 1991). Is the handedness of P11/12 migration also affected in sinistral animals? Because no mutants of left–right asymmetry are available to date in *C. elegans,* we made use of a partially sinistral mutant, called *sy466,* that we had isolated fortuitously in the species *Oscheius/*

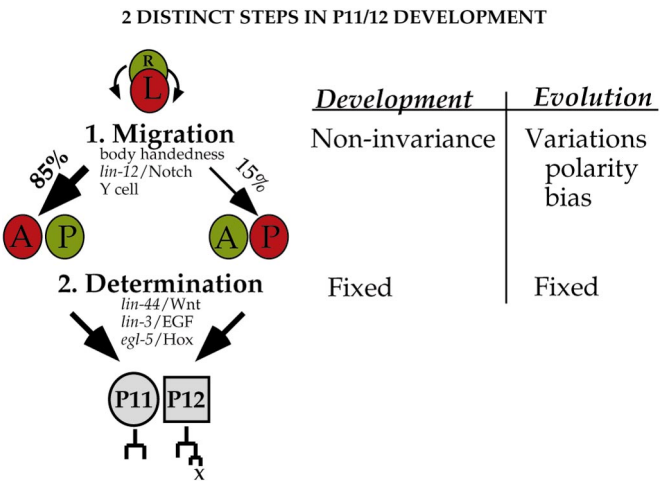

FIG. 4. Indeterminacy in the development and variations in the evolution of P11/12 migration. Migration and determination of P11/12 are two independent steps in *C. elegans* development. (1) Migration orientation is not fixed but biased, and this bias depends on body handedness, the *lin-12*/Notch receptor, and the presence of the Y cell. Left is in red, right in green. (2) Determination is fixed: the anterior cell adopts the "P11 fate" (circle), the posterior cell the "P12 fate" (square). P12 fate determination is affected by mutations in the *lin-44*/Wnt, *lin-3*/EGF, and *egl-5*/Hox pathways (Jiang and Sternberg, 1998). The orientation of P11/12 migration varies between nematode species (changes in polarity and in the presence of a bias), whereas the final fate pattern is invariant.

Dolichorhabditis sp. CEW1 (see Materials and Methods). At 25°C, 12% (n = 230) of homozygous *sy466* animals are sinistral, as determined by the positions of known asymmetric cells (see Materials and Methods). In *sy466* animals, the P12 cell usually comes from the right in dextral individuals (as in the wild type in this species), but from the left in sinistral animals (Table 1B). Handedness of P(11/12)L/R migration thus correlates with whole body handedness.

Moreover, we studied a natural sinistral population in the genus *Acrobeloides*. Most nematode species are dextral. However, we previously reported that in the species *Acrobeloides bodenheimeri* JB007 (family Cephalobidae), most animals are sinistral (Félix *et al.*, 1996). Blastomere position at the six-cell stage is reversed compared to a closely related dextral species and known asymmetric cells are inverted in larvae and adults (Félix *et al.*, 1996; De Ley *et al.*, 1999). P(11/12)L/R migration is also reversed in JB007, in comparison to closely related dextral species of the same genus (DF5049 and DF5048) (De Ley *et al.*, 1999) (Fig. 3).

In conclusion, the observed inversions in P(11/12)L/R migration in the *Oscheius/Dolichorhabditis* sp. mutant and in the sinistral species *A. bodenheimeri* both suggest that the asymmetry between the left and the right cells of the P(11/12)L/R pair is linked to whole body handedness.

Mutations in the LIN-12/Notch Receptor Affect the Orientation of P(11/12)L/R Migration but Not P11 and P12 Fate Determination

The Notch receptor is often involved in intercellular signaling between neighboring cells (Greenwald, 1998). The two *C. elegans* Notch homologs, *lin-12* and *glp-1,* are known to be involved in several left–right symmetry breakages during embryogenesis (Hutter and Schnabel, 1994, 1995; Moskowitz and Rothman, 1996; Hermann *et al.*, 2000). We wondered whether one of them could be involved in the establishment of the left–right asymmetry of P(11/12)L/R in *C. elegans,* by lateral inhibition between the two cells and/or by contact with a third cell. We studied P11/12 in *lin-12* mutants (*glp-1* mutants die as young L1 larvae before P cell migration). P11 and P12 fates are not affected in any of the mutants (or only slightly for *lin-12(n676n930);* Table 1C and Greenwald *et al.*, 1983). In contrast, P(11/12)L/R migration is clearly affected and apparently randomized in three *lin-12* reduction-of-function alleles, *n137n720* (Seydoux *et al.*, 1990), *n676n930* (Sundaram and Greenwald, 1993), and *n676n909* (Seydoux *et al.*, 1990), and in the putative null allele *n941* (Wen and Greenwald, 1999) (Table 1C). An absence of symmetry breakage between the two cells should result in an unbiased 50%–50% orientation of migration, because the two cells cannot physically migrate to the same position.

We also observed the migration of P11/12 in a *lag-2* mutant. LAG-2 is one of the four *C. elegans* homologs of the Delta protein, which is the ligand of Notch in *Drosophila* (Henderson *et al.*, 1994; Tax *et al.*, 1994). P11/12 migration is not clearly affected in this temperature-sensitive hypomorphic allele, although the bias in orientation may be reduced (Table 1C).

TABLE 2
Absence of the Y Cell Leads to an Unbiased P11/12 Migration

Genotype	Number of Y cells	Migration P12 from the right
	(A) Laser ablation	
Wild type	Y ablation	14/24 (58%)[a]
	Mock ablation	15/18
	(B) Gain-of-function allele	
lin-12(*n137*)	2	17/23
	(C) Temperature-shift experiment of a temperature-sensitive allele	
lin-12(*lf*)	None	17/33 (51%)[b]
	1	42/47 (89%)

Note. (A) Mock ablations were made on neurons located close to Y, to see if the laser heat could by itself affect the migration. (B) The orientation of P11/12 migration was scored in *lin-12*(*n137*) or *lin-12*(*n137*)/+ animals with two Y cells. (C) The temperature-sensitive *n676n930* mutant was shifted from 15 to 25°C 2–5 h before hatching (see Materials and Methods). Under these conditions, some animals have a Y cell and some do not, and P11/12 migration occurs in the absence of a functional LIN-12.

[a] Significantly different from wild-type N2 (χ^2 test, $P < 0.05$).

[b] Significantly different from N2 and from animals with 1 Y cell (χ^2 test, $P < 0.05$).

The Y Cell Is Required for the Bias in P(11/12)L/R Migration

lin-12(lf) mutants show a Y to DA9 fate transformation (Greenwald *et al.*, 1983). The Y cell is a rectal epidermal cell, positioned on the ventral midline at hatching, and DA9 is a neuron. In the *C. elegans* hermaphrodite, the Y cell later becomes a neuron (called PDA) and is replaced by the P12.pa cell as a part of the rectum wall. The Y cell is not required for P12 determination (Table 1C). Because it is very close to P11/12 (Fig. 2A), we wondered, however, whether it could play a role in P(11/12)L/R asymmetry. We ablated Y within 1 h after hatching. In the absence of Y, the bias in P(11/12)L/R migration is lost, as in *lin-12(lf)* mutants (Table 2A). Thus, the Y cell is required for the bias in P(11/12)L/R migration.

The simplest explanation is that *lin-12* is required for the determination of Y, which then biases the orientation of P(11/12)L/R migration by an unknown mechanism. The Y cell does not appear to be positioned asymmetrically at this stage. Moreover, heterozygotes or homozygotes for the dominant gain-of-function allele *lin-12(n137)* have two Y

cells that are usually positioned left–right of each other and this does not appear to have a strong effect on the orientation of P11/12 migration (Table 2B).

lin-12 could also be required twice for P11/12 migration, once in the embryo for Y cell determination and a second time for an induction by Y or a fourth cell or for lateral signaling between P(11/12)L/R. We tried to determine whether *lin-12* was required in the early larva for the bias in P11/12 migration. Using an antibody raised against GFP in a LIN-12::GFP strain (kindly provided by I. Greenwald; Levitan and Greenwald, 1998), we were unable to see any expression of the molecule in the P11/12 region in young L1 larvae, as previously described by Wilkinson and Greenwald (1995) with a *lin-12*::lac-Z strain. This favors the hypothesis that *lin-12* is required only earlier in the embryo for the determination of Y. Moreover, we made use of the *lin-12(n676n930)* temperature-sensitive allele to score animals with a Y cell in the absence of a functional LIN-12 (see Materials and Methods). As shown in Table 2C, the presence of a Y cell induces a biased orientation of migration toward P12 coming from the right, even without a functional LIN-12. In animals without a Y cell, however, the orientation of migration remains unbiased, which further demonstrates the role of the Y cell. These results strongly suggest that *lin-12* is required only once during embryogenesis for Y cell determination, which then leads to the asymmetry in the P(11/12)L/R pair.

In summary, we showed that in animals with defects in P12 fate determination, the migration of the two cells is still biased. Conversely, in the absence of the Y cell, the handedness of migration of P(11/12)L/R is affected, whereas the two cells still adopt the normal pattern of cell fates. These results show that handedness of migration and cell fate determination of P11/12 are controlled by distinct mechanisms in *C. elegans* (Fig. 4).

The Handedness of P11/12 Migration Has Frequently Changed without Fixation during Evolution of Nematodes

The orientation of P11/12 migration in *P. redivivus* (family Panagrolaimidae) was previously shown to be reversed compared to *C. elegans* (family Rhabditidae) (Sternberg and Horvitz, 1982). We wondered what the pattern of evolution of this character was and whether species with random P11/12 migration existed. P11/12 migration was observed in species of three families in the order Rhabditida, namely the Rhabditidae, the Panagrolaimidae and the Cephalobidae, and in one species of the Brevibuccidae (Fig. 3). Except for *A. bodenheimeri*, all species are dextral. We found three types of P11/12 migration: (i) highly biased like in *C. elegans*, with the posterior P12 cell usually coming from the right side; (ii) highly biased with a reversed polarity, i.e., with P12 usually coming from the left side; (iii) apparently fully random, like for other Pn cell pairs, with P12 coming from the right in around 50% of animals (DF5006 and DF5025) (Fig. 3).

Within the family Rhabditidae, we found the three possibilities. In the dextral species of the family Cephalobidae, P11/12 migration is biased toward P12 coming from the right. In contrast, in the three species of the family Panagrolaimidae and in *Brevibucca* sp. (Brevibuccidae), P11/12 migration is biased toward P12 coming from the left (Fig. 3).

DISCUSSION

Migration Handedness and Fate Determination of P11/12 Are Two Independent Developmental Processes

The biased migration of P(11/12)L and P(11/12)R defines an asymmetry between the left and the right cells of the pair. We showed that in animals with defects in P11 and P12 fate determination (mutants in the *lin-44*/Wnt and *lin-3*/EGF pathways and *egl-5* mutants), the migration of the two cells is still biased. Conversely, in sinistral animals, in *lin-12*/Notch mutants, and after Y ablation, the handedness of migration of P(11/12)L/R is clearly affected, whereas the two cells still adopt the normal pattern of cell fates (P11 fate for the anterior cell, P12 for the posterior cell). This suggests that the process controlling the chiral migration of P(11/12)L/R is independent of the fate-determination signaling pathways (Fig. 4). Also, unlike the final P11/12 fate pattern, the chirality of P(11/12)L/R migration is not fixed in a given species and varies between species.

Determination of the Handedness of P(11/12)L/R Migration by Cell Interactions

In sinistral animals, the orientation of P(11/12)L/R migration is reversed (Table 1B), like other asymmetric cell configurations (but unlike cuticle handedness; Bergmann *et al.*, 1998). We conclude that P(11/12)L/R asymmetry is a consequence of the first symmetry breakage in the six-cell stage embryo that leads to left–right asymmetry of the whole body plan (Wood, 1991). Global handedness inversion of the embryo probably results in an inversion of some cell–cell contacts that are instrumental in creating an asymmetry between P(11/12)L and P(11/12)R.

We have found that in *lin-12* mutants, the bias in the handedness of P(11/12)L/R migration is abolished (Table 1C). There are several possibilities as to the site of action of LIN-12 in a cell–cell interaction. First, LIN-12-dependent signaling plays a role in most cases of lateral inhibition between neighboring cells with a common developmental competence (Greenwald *et al.*, 1983). An obvious site of action for *lin-12* would be in a lateral inhibition between P(11/12)L/R. Second, LIN-12 is also recruited in inductive signaling between nonequivalent cells (Greenwald, 1988; Moskowitz and Rothman, 1996; Newman *et al.*, 1995; Hermann *et al.*, 2000). In the P(11/12)L/R context, it may be involved in signaling from a third cell (such as Y in the L1 stage) to one or both cells. Because LIN-12 is the receptor, it should then also be expressed in P(11/12)L or P(11/12)R.

However, we did not observe expression of LIN-12 in either cell of the P11/12 pair, at least from the time of hatching. Moreover, the Y-ablation and temperature-shift experiments indicate that *lin-12* may be required for P(11/12)L/R asymmetry only because it is required earlier to specify the Y cell (Table 2).

From the timing of the ablation experiments, the presence of Y appears to be necessary after hatching for inducing the bias in P11/12 migration. At this time, the Y cell nucleus is positioned at the midline (Fig. 2A) and apparently at a symmetric position compared to P(11/12)L and P(11/12)R, which cannot explain the bias in migration orientation. By Nomarski optics, it is difficult to distinguish cell contours and it is possible that the pattern of cell contacts is asymmetric or that Y expresses a signal asymmetrically. On the other hand, another process could be at the origin of the asymmetry of response between P(11/12)L and P(11/12)R and the Y cell would only have a permissive role in the orientation of migration.

The Handedness of P(11/12)L/R Migration Varies between Species

We determined the handedness of P11/12 migration in a wide range of free-living nematode species belonging to three closely related families (Fig. 3). Within the family Cephalobidae, the direction of the bias in P11/12 migration appears constant (P12 usually from the right), with the exception of the sinistral *A. bodenheimeri*. In the family Panagrolaimidae, the opposite handedness was found in the three species observed. The family Rhabditidae displays several intrafamily evolutionary changes: inversions in the orientation of bias as well as apparent absence of any bias. Given the known phylogeny (D. Fitch, pers. comm.; Blaxter *et al.,* 1998), the most parsimonious hypothesis is that the ancestor of all Rhabditidae had the posterior cell P12 coming from the left side and that the situation found in *C. elegans* is derived. The unresolved upper part of the tree contains species with either orientation, as well as with apparently no bias. Whether or not the unbiased situation is an intermediate, at least three changes must have occurred within the Rhabditidae (provided the position of *Rhabditis blumi* is correct). Outside the Rhabditidae, the relative positions of the three families and that of *Brevibucca* are unclear (see legend to Fig. 3), but in any configuration, at least one inversion of P11/12 handedness must have taken place between the Cephalobidae and the Panagrolaimidae and/or the basal Rhabditidae. Thus, with the inversion in *A. bodenheimeri,* at least five independent changes in P11/12 migration are likely to have occurred within the order Rhabditida during the evolution of P11/12 migration.

At which level does the evolutionary variation operate? The Y cell could be a good candidate, but we have observed the presence of a Y cell in all the species studied, even in species with an unbiased orientation of P11/12 migration (data not shown). We saw that one source of evolutionary variation in P11/12 handedness, namely the inversion in the sinistral *A. bodenheimeri,* acted very early in development, at least as early as ABa and ABp divisions. The orientation of migration also varies between dextral species (all the other species shown in Fig. 3 are dextral). One possibility is that the orientations of some cell interactions have changed during evolution of these species. Even if early cleavage handedness is the same in all dextral species, it is possible that an asymmetric cell be located on the left in one species and on the right in another, because of altered migrations in the embryo or because of a switch in fate.

The Handedness of P(11/12)L/R Migration Is Not Fixed in a Given Species

We demonstrate here a rare case of indeterminacy in the cell lineage of *C. elegans,* which concerns the handedness of migration of P(11/12)L and P(11/12)R and the resulting lineage relationship between these cells and P11/12. Strikingly, this indeterminate decision is biased (like P1/2 migration), whereas most previously described examples (the AC/VU decision, migration of other Pn cell pairs) have an unbiased 50%–50% outcome (Sulston and Horvitz, 1977).

Moreover, the handedness of P11/12 migration is not fixed in any of the 21 species in which we observed a large number of animals (10 or more) and appears unbiased in only 2 of these species (Fig. 3). Thus, surprisingly, even though the direction of the bias switches during evolution, the handedness of migration appears to remain biased but not fixed. These results on P11/12 migration can be compared and contrasted with our previous findings concerning anchor cell determination in nematodes. In *C. elegans,* either of two gonadal cells can become an anchor cell (AC) or a ventral uterine precursor (VU) and the decision is random and unbiased. In other nematodes, the AC/VU decision can be biased or fully fixed to either of them (Félix and Sternberg, 1996). Thus, whereas the full range of possibilities was found in the AC/VU case, we mostly found biased handedness for P11/12 migration (with a greater number of species studied and the same evolutionary range).

How can such a partial bias be maintained during evolution? Whichever direction P11/12 migrate, the outcome is a configuration with one anterior cell and one posterior cell. Because their determination is independent of their migration, these cells adopt the P11 and P12 fates, respectively, regardless of their left–right origin. Whereas having a cell with a P12 fate is likely to be important for correct rectum formation, the handedness of the cell migration may not be under strong selection pressure. In other words, the final P11/12 cell fate pattern matters to the animal, but probably not the developmental route (the handedness of migration) leading to it (Fig. 4).

Where does this indeterminacy in the handedness of migration stem from during development? The strains we observed are unlikely to be genetically heterogeneous, especially those that are hermaphroditic or parthenogenetic. Thus, the indeterminacy of P11/12 development is not the

result of a genetic heterogeneity in the observed populations. If local cell interactions in the posterior region are required for determination of the handedness of migration, it is possible that the position of some signaling cell(s) may vary between individuals. We observed the positions of cells in the posterior region in some *C. elegans* individuals with the unusual handedness of migration, but failed to find unusual positions of obvious candidate cells (such as Y or left–right asymmetric cells). We may have missed asymmetric cells or earlier embryonic asymmetries. Alternatively, the indeterminacy may be the result of a variable interpretation of a weakly, but consistently, asymmetric signal. Note that the mechanisms leading to variations in the orientation of P11/12 migration between species are not necessarily the same as those leading to its indeterminacy in a given species.

ACKNOWLEDGMENTS

We are very grateful to P. Sternberg, I. Greenwald, and L. Jiang for discussions and to D. Fitch for communication of unpublished results. We thank an anonymous reviewer for helpful comments on the manuscript. We thank G. Maro for help with characterization of *sy466* and L. Carta, D. Fitch, P. De Ley, J. Baldwin, W. Sudhaus, P. Sternberg, I. Greenwald, and the *C. elegans* Genetics Center (NIH) for strains. M.D. was supported by a Ph.D. fellowship from the ARC and the FRM. Work in the laboratory of M.-A.F. is supported by the CNRS, the FRM, and the ARC.

REFERENCES

Andrassy, I. (1984). "Klasse Nematoda." Gustav Fischer Verlag, Stuttgart.

Austin, J., and Kimble, J. (1987). *glp-1* is required in the germ line for regulation of the decision between mitosis and meiosis in *C. elegans*. *Cell* **51,** 589–599.

Bargmann, C. I., and Avery, L. (1995). Laser killing of cells in *Caenorhabditis elegans*. *In* "*Caenorhabditis elegans:* Modern Biological Analysis of an Organism" (H. F. Epstein and D. C. Shakes, Eds.), pp. 225–250. Academic Press, San Diego.

Bergmann, D. C., Crew, J. R., Kramer, J. M., and Wood, W. B. (1998). Cuticle chirality and body handedness in Caenorhabditis elegans. *Dev. Genet.* **23,** 164–174.

Blaxter, M. L., De Ley, P., Garey, J. R., Liu, L. X., Scheldeman, P., Vierstraete, A., Vanfleteren, J. R., Mackey, L. Y., Dorris, M., Frisse, L. M., Vida, J. T., and Thomas, W. K. (1998). A molecular evolutionary framework for the phylum Nematoda. *Nature* **392,** 71–75.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77,** 71–94.

De Ley, P., Félix, M.-A., Frisse, L. M., Nadler, S. A., Sternberg, P. W., and Thomas, W. K. (1999). Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology* **1,** 591–612.

Eisenmann, D. M., Maloof, J. N., Simske, J. S., Kenyon, C., and Kim, S. K. (1998). The β-catenin homolog BAR-1 and LET-60 Ras coordinately regulate the Hox gene *lin-39* during *Caenorhabditis elegans* vulval development. *Development* **125,** 3667–3680.

Félix, M.-A., Sternberg, P. W. (1996). Symmetry breakage in the development of one-armed gonads in nematodes. *Development* **122,** 2129–2142.

Félix, M.-A., Sternberg, P. W., and De Ley, P. (1996). Sinistral nematode population. *Nature* **381,** 122.

Félix, M.-A., Delattre, M., and Dichtel, M.-L. (2000a). Comparative developmental studies using *Oscheius/Dolichorhabditis* sp. CEW1 (Rhabditidae). *Nematology* **2,** 89–98.

Félix, M. A., De Ley, P., Sommer, R. J., Frisse, L., Nadler, S. A., Thomas, W. K., Vanfleteren, J., and Sternberg, P. W. (2000b). Evolution of vulva development in the Cephalobina (Nematoda). *Dev. Biol.* **221,** 68–86.

Ferguson, E., and Horvitz, H. R. (1985). Identification and characterization of 22 genes that affect the vulval cell lineages of *Caenorhabditis elegans*. *Genetics* **110,** 17–72.

Greenwald, I. (1998). LIN-12/Notch signaling: Lessons from worms and flies. *Genes Dev.* **12,** 1751–1762.

Greenwald, I. S., Sternberg, P. W., and Horvitz, H. R. (1983). The *lin-12* locus specifies cell fates in *Caenorhabditis elegans*. *Cell* **34,** 435–444.

Hedgecock, E. M., and Herman, R. K. (1995). The *ncl-1* gene and genetic mosaics of *Caenorhabditis elegans*. *Genetics* **141,** 989–1006.

Henderson, S. T., Gao, D., Lambie, E. J., and Kimble, J. (1994). *lag-2* may encode a signaling ligand for the GLP-1 and LIN-12 receptors of *C. elegans*. *Development* **120,** 2913–2924.

Herman, M. A., and Horvitz, H. R. (1994). The *Caenorhabditis elegans* gene *lin-44* controls the polarity of asymmetric cell divisions. *Development* **120,** 1035–1047.

Herman, M. A., Vassillieva, L. L., Horvitz, H. R., Shaw, J. E., and Herman, R. K. (1995). The *C. elegans* gene *lin-44,* which controls the polarity of certain asymmetric cell divisions, encodes a Wnt protein and acts cell nonautonomously. *Cell* **83,** 101–110.

Hermann, G. J., Leung, B., and Priess, J. R. (2000). Left–right asymmetry in *C. elegans* intestine organogenesis involves a LIN-12/Notch signaling pathway. *Development* **127,** 3429–3440.

Hodgkin, J., Horvitz, H. R., and Brenner, S. (1979). Nondisjunction mutants of the nematode *Caenorhabditis elegans*. *Genetics* **91,** 67–94.

Hutter, H., and Schnabel, R. (1994). *glp-1* and inductions establishing embryonic axes in *C. elegans*. *Development* **120,** 2051–2064.

Hutter, H., and Schnabel, R. (1995). Establishment of left–right asymmetry in the *Caenorhabditis elegans* embryo: A multistep process involving a series of inductive events. *Development* **121,** 3417–3424.

Jiang, L. I., and Sternberg, P. W. (1998). Interactions of EGF, Wnt and HOM-C genes specify the P12 neuroectoblast fate in *C. elegans*. *Development* **125,** 2337–2347.

Lambie, E. J., and Kimble, J. (1991). Two homologous regulatory genes, *lin-12* and *glp-1,* have overlapping functions. *Development* **112,** 231–240.

Levitan, D., and Greenwald, I. (1998). LIN-12 protein expression and localization during vulval development in *C. elegans*. *Development* **125,** 3101–3109.

Liu, J., Tzou, P., Hill, R. J., and Sternberg, P. W. (1999). Structural requirements for the tissue-specific and tissue-general functions of the *C. elegans* epidermal growth factor LIN-3. *Genetics* **153,** 1257–1269.

Moerman, D. G., and Baillie, D. L. (1979). Genetic organization in *Caenorhabditis elegans:* Fine-structure analysis of the *unc-22* gene. *Genetics* **91,** 95–104.

Moskowitz, I. P. G., and Rothman, J. H. (1996). *lin-12* and *glp-1* are required zygotically for early embryonic cellular interactions and are regulated by maternal GLP-1 signaling in *Caenorhabditis elegans*. *Development* **122,** 4105–4117.

Newman, A. P., White, J. G., and Sternberg, P. W. (1995). The *C. elegans lin-12* gene mediates induction of ventral uterine specialization by the anchor cell. *Development* **121,** 263–271.

Rogalski, T. M., and Baillie, D. L. (1985). Genetic organization of the *unc-22* gene and the adjacent region in *Caenorhabditis elegans*. *Mol. Gen. Genet.* **201,** 409–414.

Sawa, H., Lobel, L., and Horvitz, H. R. (1996). The *Caenorhabditis elegans* gene *lin-17*, which is required for certain asymmetric cell divisions, encodes a putative seven-transmembrane protein similar to the *Drosophila* Frizzled protein. *Genes Dev.* **10,** 2189–2197.

Seydoux, G., and Greenwald, I. (1989). Cell autonomy of *lin-12* function in a cell fate decision in *C. elegans*. *Cell* **57,** 1237–1245.

Seydoux, G., Schedl, T., and Greenwald, I. (1990). Cell–cell interactions prevent a potential inductive interaction between soma and germline in *C. elegans*. *Cell* **61,** 939–951.

Sharma-Kishore, R., White, J. G., Southgate, E., and Podbilewicz, B. (1999). Formation of the vulva in *Caenorhabditis elegans:* A paradigm for organogenesis. *Development* **126,** 691–699.

Sternberg, P. W., and Horvitz, H. R. (1982). Postembryonic nongonadal cell lineages of the nematode *Panagrellus redivivus:* Description and comparison with those of *Caenorhabditis elegans*. *Dev. Biol.* **93,** 181–205.

Sudhaus, W., and Fitch, D. (2001). Comparative studies on the phylogeny and systematics of the Rhabditidae (Nematoda). *J. Nematol.*, in press.

Sulston, J., and Horvitz, H. R. (1977). Postembryonic cell lineages of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **56,** 110–156.

Sulston, J. E., Schierenberg, E., White, J. G., and Thomson, J. N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **100,** 64–119.

Sulston, J. E., and Hodgkin, J. (1988). Methods. *In* "The Nematode *Caenorhabditis elegans*" (W. Wood, Ed.), pp. 587–606. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sundaram, M., and Greenwald, I. (1993). Genetic and phenotypic studies of hypomorphic *lin-12* mutants in *Caenorhabditis elegans*. *Genetics* **135,** 755–763.

Tax, F. E., Yeargers, J. J., and Thomas, J. H. (1994). Sequence of *C. elegans lag-2* reveals a cell-signalling domain shared with *Delta* and *Serrate* of *Drosophila*. *Nature* **368,** 150–154.

Trent, C., Tsung, N., and Horvitz, H. R. (1983). Egg-laying defective mutants of the nematode *Caenorhabditis elegans*. *Genetics* **104,** 619–647.

Wen, C., and Greenwald, I. (1999). p24 proteins and quality control of LIN-12 and GLP-1 trafficking in *Caenorhabditis elegans*. *J. Cell Biol.* **145,** 1165–1175.

Wilkinson, H. A., and Greenwald, I. (1995). Spatial and temporal patterns of *lin-12* expression during *C. elegans* hermaphrodite development. *Genetics* **141,** 513–526.

Wood, W. B. (1991). Evidence from reversal of handedness in *C. elegans* for early cell interactions determining cell fates. *Nature* **349,** 536–538.

zur Strassen, O. (1951). Der Erbgang der Nematoden-Asymmetrie. *Verh. Dtsch. Zool. Ges.* 77–81.

Received for publication September 26, 2000
Accepted January 2, 2001
Published online March 13, 2001